685-

BIOCHEMICAL OXIDATION OF DAIRY WASTES

III. Failure of Sodium Nitrate as a Source of Oxygen *

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In the course of investigations on the biochemical oxidation of dairy waste, the use of sodium nitrate as a source of oxygen in disposal of dairy waste was suggested. Lagoons (7) and streams (4) have shown beneficial effects from nitrate. Such treatment would be desirable for the prevention of offensive odors associated with anaerobic conditions when mechanical failure in a milk plant prevents aeration of the sludge-waste mixture.

This report shows that utilizing sodium nitrate as a source of oxygen for such a purpose is not feasible. The results were obtained by several methods. The Warburg apparatus was used in one phase of the study to determine the oxygen uptake and carbon dioxide evolved. In another part of the experiment, simple jars containing sludge and skim milk were aerated with carbon dioxide-free air or nitrogen for a number of hours (5). The carbon dioxide liberated was measured. It was also determined in mixtures of sludge and skim milk containing sodium nitrate that had been left undisturbed for several days.

Earlier studies on simulated dairy waste showed that skim milk or its constituents (lactose and casein) are readily available to microorganisms in aerated sludge (1). An organic solids balance established on the influent and effluent during a continuous aeration study demonstrated that about 50 per cent of the organic matter was oxidized and the growth of the cells assimilated the remainder (3). Manometric methods using the Warburg technique were applied to determine the rate and amount of oxidation (1). These experiments demonstrated that skim milk and its two principal components had a high rate of oxidation for a few hours, but the rate was reduced to that of the control in 6 hr. At this time, 32 to 40 per cent of the substrate was oxidized. Later the application of polarographic procedures for measuring oxygen in solution showed oxygen disappearance was rapid under anaerobic conditions (2). Only a few minutes were required for oxygen depletion when 1,000 p.p.m. skim milk were added to a pre-aerated sludge. When the waste (skim milk) was less concentrated, a longer time was required to deplete the oxygen of the sludge solution. The data indicated that the rate of oxidation remained fairly constant until the concentration of oxygen in solution was less than 0.5 p.p.m.

Procedures

It had already been shown that an aerated sludge system containing skim milk, lactose, or casein had a respiratory quotient of unity (1). This indicated that the volume of CO_2 evolved was equal to the volume of oxygen consumed; that is, R.Q. = CO_2/O_2. Thus, measuring the CO_2 evolved from a system gave a measure of the oxidation

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of the added material. The amounts of CO_2 formed in the experiments in the study reported here were determined manometrically and titrimetrically on the same sludge-substrate mixtures.

Direct Manometric Determination of CO_2

Three milliliters of aerated sludge were placed in a Warburg vessel. Into the center well was introduced 0.3 ml. 3*N* H_2SO_4 and 0.3 ml. 10 per cent KOH was put in the side arm. Substrate in the amount of 0.3 ml. was added to the sludge, and the vessel was attached to the manometer. After equilibration at 30° C. for 15 min., all vessel outlets were closed. The contents of one set of flasks were immediately mixed thoroughly, and the reading was recorded. A second set of flasks was permitted to oscillate until sufficient oxygen was used by the sludge. The contents were mixed as before. The difference in reading from that at the start gave the amount of CO_2 evolved.

Vessels were also equilibrated with nitrogen gas. Results were summarized as μl. CO_2 evolved per 3 ml. of sludge for the period of time indicated in each experiment.

Indirect Manometric Estimation of CO_2

Since the R.Q. of the substrates under study was equal to unity, the CO_2 produced and retained by the KOH in the vessels was equal in volume to the oxygen consumed. The oxygen uptake was determined as described in "Standard Methods" (8). As before, 3 ml. of sludge were placed in a Warburg vessel. The 0.3 ml. of substrate was kept separate by adding it in the side arm; 0.3 ml. of 10 per cent KOH was the absorbent in the center well.

After equilibration with air, the outlets were closed, and the skim milk and sludge were mixed. The oxygen uptake was determined over a period of 6 hr. The equivalent weights of CO_2 produced hourly were calculated by use of the appropriate factor; that is, 1 g. O_2 = 1.375 g. CO_2.

Titrimetric Determination of CO_2

The preceding determinations required small quantities of materials and special technics applicable to the Warburg apparatus. Such difficulties may be obviated for plant application by use of larger quantities and simple titrations (5). Five hundred ml. of sludge were placed in the jar and pre-aerated for a time equal to the equilibration period used in the Warburg study. Fifty ml. of the substrate were then added through the separatory funnel, and the CO_2 was collected. CO_2-free air was passed through the sludge-substrate mixture. The spent air was then bubbled through 0.1 *N* $Ba(OH)_2$, which trapped the CO_2; the excess $Ba(OH)_2$ was titrated with 0.1 *N* oxalic acid. The difference between the amount of $Ba(OH)_2$ present at the start and that found by oxalic acid titration was the amount combined with the CO_2; 1 ml. of 0.1 *N* $Ba(OH)_2$ = 2.2 mg. of CO_2. These experiments were conducted for a period of 6 hr. and a determination was made every hour.

In all experiments except one, in which the rate of air was varied, the rate of air flow was 500 ml. per minute; that is, 1 volume of air per volume of solution per minute. Nitrogen gas was also used at the same rate of flow.

Chemical Oxygen Consumed

The chemical oxygen consumed values of the total sludge, the supernatant liquor, and the substrates were determined by the rapid method (6).

Source of Sludge

The mixed sludge culture was obtained from a modified aerobic fermentor after several weeks' propagation (3). During this time, a 0.1 per

TABLE I.—Substrate Composition, Oxygen Requirement, and $NaNO_3$ Supplementation

Substrate		Oxygen Demand		$NaNO_3$ Added (p.p.m.)	Oxygen Available (p.p.m.)
Type	(p.p.m.)	Theor. (p.p.m.)	Observed (p.p.m.)		
Skim milk	10,000	11,430	3,570	10,130	5,720
Lactose	5,000	5,300	1,960	4,690	2,650
Casein	3,500	4,790	1,910	4,250	2,400

cent solution of dry skim milk was added continually at the rate of 1 l. per hour to the 20 l. in the fermentor.

Substrate Mixtures

In these CO_2 evolution studies, one part of the concentrated substrate (Table I) was added to 10 parts of sludge. Thus, 0.3 ml. was added to the 3 ml. in the Warburg vessels and 50 ml. to the 500 ml. of sludge in the aeration jars. The resulting mixtures gave the desired concentration of organic matter, as measured by the oxygen consumed.

Addition of Sodium Nitrate

The amount of $NaNO_3$ required to supply oxygen was calculated from earlier data showing oxygen utilization by various nutrients (1). Fifty per cent of the theoretical quantity required for complete oxidation was added. This was in excess of the requirement for the observed oxidation of each substrate (Table I). It was assumed that all the oxygen in the nitrate was available. Solutions of skim milk, lactose, and casein were prepared with and without the calculated amount of $NaNO_3$. Thus, the same material was used for each series of tests.

Experimental

Oxidation of Skim Milk

Table II shows results of direct manometric determinations of CO_2 in 3-ml. sludge samples to which 0.3 ml. of milk solution had been added. This Warburg test was terminated in 2.5 hr., for by this time sufficient CO_2 was produced. The importance of an oxygen supply was clearly indicated, as only about one-fifth as much CO_2 was evolved under nitrogen gas. Addition of $NaNO_3$ did not increase CO_2 production in this period of time.

Figure 1 shows the weight of CO_2 produced during a 6-hr. period when CO_2-free air and nitrogen were passed through the aerators, each containing 500 ml. sludge and 50 ml. milk solution. Simultaneously, an oxygen consumption test was made with the Warburg apparatus. The weight of CO_2 was calculated, and the values obtained by the two methods were practically identical. Addition of $NaNO_3$ had no marked influence upon the oxidation of skim milk. The amount of CO_2 evolved was only slightly greater than that ob-

TABLE II.—Carbon Dioxide Evolved from 3 Ml. of Sludge and 0.3 Ml. of Substrate

Substrate	Time (hr.)	CO_2 Evolved in Presence of			
		Air (μl.)	Air + $NaNO_3$ (μl.)	N_2 (μl.)	N_2 + $NaNO_3$ (μl.)
Skim milk, 1.0%	2.5	335	334	59	68
Lactose, 0.5%	4	368	361	142	135
Casein, 0.35%	6	309	279	13	4

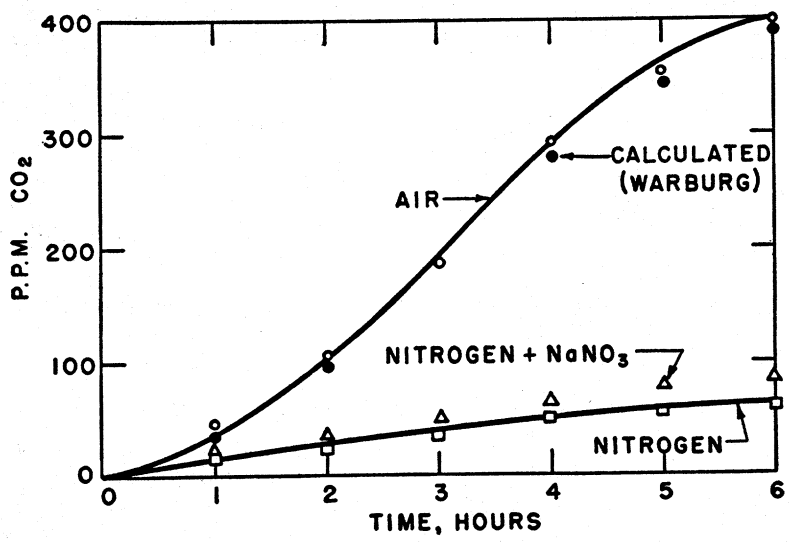

FIGURE 1.—Evolution of CO_2 from sludge-skim milk mixture.

tained when nitrogen gas alone was used, although as much as 4,000 p.p.m. $NaNO_3$ were added in some tests. Earlier tests showed that $NaNO_3$ in the presence of air had no effect.

At the termination of this experiment, the chemical oxygen consumed determinations of the contents of each flask were made and compared with the values originally present (Table III). After 6-hr. aeration, practically all solubles were gone; about 33 per cent was completely oxidized, and the remainder was in solid form. The mixtures through which nitrogen gas bubbled showed considerably less activity. Although there was some formation of solids, about two-thirds of the soluble portion still remained. The pH at the end was 7.0 in the aerated solution, 5.1 in nitrogen gas, and 5.3 in the solution having $NaNO_3$. It was obvious that the oxygen of $NaNO_3$ was unable to replace the oxygen of the air in sludge-skim milk mixtures.

Oxidation of Lactose

Similar experiments were conducted with a buffered solution containing 0.5 per cent lactose. This concentration gave the desired amount of lactose in the mixture, since one-half the solids of the simulated dairy waste consisted of this sugar. Other tests conducted with unbuffered solutions gave almost identical results.

After 4 hr., sufficient CO_2 was formed in the Warburg vessel to give the data shown in Table II. The activity in the presence of nitrogen gas was only two-fifths of that in air. Addition of $NaNO_3$ did not increase CO_2 production; hence, nitrate-oxygen was not used for this purpose in this period of time.

The quantities of CO_2 evolved in 6 hr. by 500 ml. of sludge containing lactose are plotted in Figure 2. At the same time, oxygen uptake was de-

TABLE III.—Chemical Oxygen Consumption of Sludge-Skim Milk Mixtures in Presence of Air, Nitrogen, and $NaNO_3$

Environment	Total (p.p.m.)	Solubles (p.p.m.)	Solids (p.p.m.)	Oxidized (p.p.m.)
—[1]	1,336	996[2]	340	0
Air[3]	1,001	62	939	335
N_2[3]	1,237	602	635	99
N_2+NaNO_3[3]	1,237	684	553	99

[1] Original substrate.
[2] Sludge solubles 38 p.p.m., milk 958 p.p.m.
[3] After 6 hr. of treatment.

TABLE IV.—Chemical Oxygen Consumption of Sludge-Lactose Mixtures in Presence of Air, Nitrogen, and $NaNO_3$

Environment	Total (p.p.m.)	Solubles (p.p.m.)	Solids (p.p.m.)	Oxidized (p.p.m.)
—[1]	899	581[2]	318	0
Air[3]	690	116	574	209
Air+$NaNO_3$[3]	650	116	534	249
N_2[3]	806	505	318	93
N_2+$NaNO_3$[3]	796	504	318	103

[1] Original substrate.
[2] Sludge solubles 70 p.p.m., lactose 511 p.p.m.
[3] After 6 hr. of treatment.

termined manometrically. The CO_2 obtained from the aerated mixture was slightly less than the amount calculated from the oxygen uptake in the Warburg vessel. However, the slope of the lines shows that the rate of oxidation was practically alike. Activity was retarded under nitrogen gas to about one-seventh of that in the presence of air. Addition of nitrates did not increase activity.

The analytical data in Table IV show the failure of $NaNO_3$ as an oxidizing agent in this experiment. This salt did not aid formation of solids or gas from lactose.

Oxidation of Casein

The solids in simulated dairy waste contained about 35 per cent protein. Therefore, a series of experiments was conducted with a casein substrate. In the absence of air practically no CO_2 was liberated, and addition of $NaNO_3$ did not help (Table II). Liberation of CO_2 was much slower than with the previous two substrates, requiring 6 hr. to obtain substantial differences.

In tests using casein, a difficulty occurred repeatedly. The oxygen uptake in the Warburg vessel showed that about twice as much CO_2 was produced as was obtained from the jar aerator. Apparently, the alkaline condition brought about by the oxidation of the casein held CO_2 in solution, and it was not passed into the $Ba(OH)_2$. This difficulty was overcome in experiments, in which H_2SO_4 to about pH 3 was added to the sludge-casein mixture at the end of the aeration period (5). Aeration was continued for 30 min., a fresh set of tubes being used with $Ba(OH)_2$. The amount of CO_2 evolved after acidification was about equal to the amount evolved during the experiment. Apparently, application of this test to proteins would require liberation of the CO_2 by acidification after the aeration period.

Decreasing Air Flow

The quantity of air used in these studies was in great excess in order to assure good agitation, thorough aeration, and an excess of oxygen. A study with decreasing rates of air flow was made in which the jar aerators containing 500 ml. sludge and skim milk were used. Four rates were tested—1, ½, ¼, and ⅛ volume of air per volume of sludge mixture per minute. Production of CO_2 was almost the same at these four flow rates; the total amount of CO_2 ranged from 335 to 320 p.p.m., with decreasing air flow after 6-hr. aeration.

$NaNO_3$ under Quiescent Conditions

Agitation of the mixtures in the foregoing tests may have resulted in adverse utilization of the nitrate oxygen. Further, as interest centered on the immediate effect, sufficient time was not allowed for possible use. Therefore, sludge-milk mixtures in two bottles having water traps were left undisturbed for 10 days. Each bottle had 3 l. aerated sludge and 1 l. solution containing 0.1 per cent dried skim milk. To one was added 3 g. $NaNO_3$, giving a concentration of 125 p.p.m. nitrogen, or 430 p.p.m. oxygen. At 24-hr. intervals, samples of supernatant solution were cautiously withdrawn with a pipette held 1 in. below the surface. Nitrates, nitrites, and oxygen

consumed * were determined (Figure 3).

In 24 hr., the oxygen consumed value of the supernatant was half that of the complete mixture at the start. A 2-hr. sample had shown only 10 per cent reduction. At these periods, the $NaNO_3$ had a possible adverse effect on the oxidation of the organic matter. After 7 or 10 days, there was a slight favorable action. About one-half the nitrate was changed to nitrite within 24 hr.; both were gone in 5 days. Although the nitrate was used, CO_2 or other gases did not bubble through the water trap. Addition of the $NaNO_3$ was of no help in controlling disagreeable odors.

Discussion

These experiments showed that $NaNO_3$ has no value as an oxidizing agent in the disposal of dairy wastes, especially for emergency purposes when oxidizing agents must be added to avoid odors. An oxidizing agent must have an immediate effect, because addition of dairy waste to a sludge causes anaerobic conditions in a few minutes. The rate of CO_2 produced by a sludge-skim milk mixture in 6 hr. remained unchanged by the addition of $NaNO_3$. Similar negative results were obtained when the components of skim milk were used as substrates.

On the other hand, compressed oxygen may offer a means for maintaining aerobic conditions. Such oxygen is usually easily available and should find a use for odor abatement in case of mechanical failure. Investigations are under way on use of compressed oxygen for this purpose.

Addition of nitrates was of no value during the 10-day period. The nitrates disappeared, but the disagreeable anaerobic conditions persisted. Apparently free oxygen was not made available by the microorganism to oxidize odor-producing substances. It seemed that in the presence of large amounts of organic nitrogeneous matter sufficient nitrogen for cell activity existed and the nitrate-nitrogen was not needed; hence, oxygen was not liberated.

In high carbohydrate-containing wastes, on the other hand, the nitrogen of added nitrates may be used for bacterial synthesis. All the oxygen may not be needed for growth. In that case, the excess oxygen may be diffused in the liquid and serve as a means of oxidizing malodorous substances in the same manner as in lagoons. Unfortunately, dairy wastes cannot utilize nitrates for this purpose, as such a waste contains the nutrients in balanced proportions for microbial growth.

Summary

Oxidation of skim milk, casein, and lactose by aerated sludge was determined by measuring the CO_2 evolved titrimetrically and manometrically. Evolution of CO_2, and hence the activity of the sludge, was not markedly increased by addition of $NaNO_3$. The nitrate-oxygen was not used as an oxygen source by the sludge-waste mixture, possibly because the high organic nitrogen content of the mixture did not favor the growth of desired microorganisms.

Acknowledgment

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* In Figure 3 this value is called "chemical oxygen demand (C.O.D.)"

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